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APPENDIX 1

APPLICANTS	:	ALAN S. KOPIN MARTIN BEINBORN
TITLE	:	ASSAYS FOR IDENTIFYING RECEPTORS HAVING ALTERATIONS IN SIGNALING

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G Protein Chimera Users Manual

Introduction

Since the first description of G protein chimeras that can alter the signaling phenotype of receptors, many investigators have found them useful for a variety of research purposes. Several people who work with G_i-coupled receptors have found that it is easier to study the stimulation of phospholipase C than the inhibition of adenylyl cyclase. Several groups have used the chimeras to develop rapid assays of receptor activation that can be used for screening mutants or agonist drugs. Others have used the chimeras to complement mutant receptors in detailed structure-function studies.

Over the past four years, I have sent over sixty samples of chimeras. The collection of chimeras has gradually grown and has been improved by the addition of epitope-tagged versions. This update should help people use the chimeras most effectively. Many people who received the original clones may want to upgrade to the new versions.

Structure-Function Studies with G Proteins

The carboxyl-terminus of the G alpha protein subunit is a key determinant of receptor specificity. We have previously shown that the Gq alpha subunit (alpha q) can be made to respond to Gi alpha-coupled receptors by replacing its carboxyl-terminus with the corresponding Gi2 alpha, Go alpha, or Gz alpha residues (2). We have recently extended these findings in three ways: 1.C-terminal mutations of Gq alpha/Gi alpha chimeras show that the critical amino acids are in the -3 and -4 positions. 2.Exchange of carboxyl-termini between Gq alpha and Gs alpha allows activation by receptors appropriate to the C-terminal residues. 3.We identify receptors that either do or do not activate the expected C-terminal chimeras (Gq alpha/Gi alpha, Gq alpha/Gs alpha, Gs alpha/Gq alpha). Replacement of the five carboxyl-terminal amino acids of Gq alpha with the Gs alpha sequence permitted an Gs alpha-coupled receptor (the V2 vasopressin receptor, but not the beta 2-adrenoceptor) to stimulate phospholipase C. Replacement of the five carboxyl-terminal amino acids of Gs alpha with residues of Gq alpha permitted certain Gq alpha-coupled receptors (bombesin and V1a vasopressin receptors, but not the Oxytocin receptor) to stimulate adenylyl cyclase. Thus, the relative importance of the G alpha carboxyl-terminus for permitting coupling to a new receptor depends on the receptor with which it is paired. These studies refine our understanding of the basis of receptor-G alpha specificity. Substitutions of the C-termini of Gq alpha and other G-alpha subunits has recently been instrumental in developing high throughput screens for new agonists of G protein-coupled receptors [Broach J.R. and Thorner J. (1996) High-throughput screening for drug discovery. *Nature* **384** (Suppl.):14-16].

Chimera Summary

Notes on the chimeras:

1. All have been subcloned into pcDNA-1, in the Bam HI/Nsi I cassette with q4WT as parent construct for the "q" chimeras and Gs-WT-HA as the parent construct for the "s" chimeras (see below for the description of the parent constructs).

2. All have the internal HA epitope, which does not affect receptor coupling, yet allows recognition

by the 12CA5 antibody (available from Boehringer Mannheim as a purified monoclonal and directly conjugated to HRP, which is convenient for Westerns).

3. All the constructs are in pcDNA-1 ,which require sup F selection for Amp and Tet resistance. This requires special competent bacteria that are available in most labs, but can also be purchased from Invitrogen (for example, mc1061/p3).

- **qi5-HA:** This is Gq alpha with the C-terminal amino acids changed from Gq alpha to Gi alpha residues (EYNLV to DCGLF). This construct allows many Gi-coupled receptors to stimulate phospholipase C (PLC). This is the most popular chimera, perhaps because it is easier to talk about coupling to Gi-coupled receptors with "qi5" rather than "qo5" or "qz5."

  Click here to see sequence for qi5.

- **qo5-HA:** This is Gq alpha with the C-terminal amino acids changed from Gq alpha to Go alpha residues (EYNLV to GCGLY). Works the same as qi5 but (for unknown reasons) has a slightly lower basal PLC activity. This can increase the signal-to-noise ratio, so I tend to use it the most.

  Click here to see sequence for qo5.

- **qz5-HA:** This is Gq alpha with the C-terminal amino acids changed from Gq alpha to Gz alpha residues (EYNLV to YIGLC). Works the same as qi5 and is the least popular since no one knows what Gz alpha really does in nature. Since qz5 is not sensitive to pertussis toxin, qz5 may be the only G protein activated by a Gi-coupled receptor in cells treated with pertussis toxin. This trick can be experimentally useful in settings where you want experimental control of the exact G protein and the receptor that is activated. It is theoretically possible that this construct will work better than the other constructs for particular receptors, but I have not seen this happen yet.

- **qs5-HA:** This is Gq alpha with the C-terminal amino acids changed from Gq alpha to Gs alpha residues (EYNLV to QYELL). This construct allows some Gs-coupled receptors to stimulate phospholipase C.

  Click here to see sequence for qs5.

- **sq5-HA:** This is Gs alpha with the C-terminal amino acids changed from Gs alpha to Gq alpha residues (QYELL to EYNLV). This construct allows some Gq-coupled receptors to stimulate Adenylate cyclase. There isn't much experience with this chimera at the moment. It may be useful for people who find the AC stimulation is better readout of receptor activation than PLC stimulation.

- **13Z:** This is G13 alpha with the C-terminal amino acids changed from G13 alpha to Gz alpha residues (QLMLQ to YIGLC). This construct allows some Gi-coupled receptors to stimulate an increase in pH of cells. There isn't much experience with this chimera at the moment, but it was used successfully with the D2-dopamine receptor [see Voyno-Yasenetskaya et al. (1994) *JBC* **269**:4721-4724].

  Please note that this construct is not epitope tagged since no one has made a reliable internal

tag for G13 alpha.

Parent Constructs:

q4WT: This is Gq alpha with an HA epitope engineered into an internal site that does not seem to affect receptor coupling in multiple studies. Epitope tagged by Paul Wilson, see Wedegaertner, *JBC* **268**: 25001-25008. The 5" non-coding sequences were removed, but the 3' non-coding sequences remain, as in Strathmann & Simon (1990) *PNAS* **87**:9113-9117. The parent construct has been donated to the ATCC by the Bourne Lab.

Click here to see sequence for q4WT

Gs-WT-HA: This construct is also known as "GSL" in the Bourne Lab. This is "wild type" Gs alpha in pcDNA-1 with a HA epitope engineered into an internal site that does not seem to affect receptor coupling in multiple studies. [See Levis & Bourne (1992) *J. Cell Biol.* **119**:1297-1307] The parent construct has been donated to the ATCC by the Bourne Lab.

Click here to see sequence for Gs-WT-HA.

Below is a list of publications that describes how we have used the G alpha C-terminal chimeras:

Initial description of chimeras:

1. Conklin B.R., Farfel Z., Lustig K.D., Julius D. and Bourne H.R. (1993) Substitution of three amino acids switches receptor specificity of Gq alpha to that of Gi alpha. *Nature* **363**:274-276

2. Conklin B.R., Herzmark P., Ishida S., Voyno-Yasenetskaya T.A., Sun Y. and Bourne H.R. (1996) C-Terminal mutations of Gq alpha and Gs alpha that alter the fidelity of receptor activation. *Mol. Pharmacol.* **50**:885-890.

3. Voyno-Yasenetskaya T., Conklin B.R., Gilbert R.L., Hooley R., Bourne H.R. and Barber D.L. (1994) G13 alpha stimulates Na-H Exchange. *J. Biol. Chem.* **269**:4721-4724.

Chimeras used in recent studies:

1. Liu J., Conklin B.R., Blin N., Yun J., Wess J. (1995) Identification of a receptor/G-protein contact site critical for signaling specificity and G-protein activation. *Proceedings of the National Academy of Sciences, U.S.A.* **92**:11642-11646.

2. Messier T.L., Dorman C.M., Bräuner-Osborne H., Eubanks D. and Brann M.R. (1995) High throughput assays of cloned adrenergic, muscarinic, neurokinin, and neurotrophin receptors in living mammalian cells. *Pharmacol. Toxicol.* **76**:308-311.

3. Liu J., Blin N., Conklin B.R. and Wess J. (1996) Molecular mechanisms involved in muscarinic acetylcholine receptor-mediated G protein activation studied by insertion mutagenesis. *J. Biol. Chem.* **271**:6172-6178 .

4. Boss V., Talpade D.J. and Murphy T.J. (1996) Induction of NFAT-mediated transcription by Gq-coupled receptors in lymphoid and non-lymphoid cells. *J. Biol. Chem.* **271**:10429-10432.

5. Arai H. and Charo I.F. (1996) Differential regulation of G-protein-mediated signaling by chemokine receptors. *J. Biol. Chem.* **271**:21814-21819

6. Liu J., Blin N., Conklin B.R. and Wess J. (1996) Molecular mechanisms involved in muscarinic acetylcholine receptor-mediated G protein activation studied by insertion mutagenesis, *J. Biol. Chem.* **271**:6172-6178.

7. Gomeza J., Mary S., Brabet I., Parmentier M.-L, Restituito S., Bockaert J. and Pin J.-P. (1996) Coupling of metabotropic glutamate receptors 2 and 4 to G15 alpha, G16 alpha, and chimeric Gqalpha/Gi alpha proteins: Characterization of new antagonists. *Mol. Pharmacol.* **50**:923-930.

8. Parmentier M.-L., Pin J.-P., Bockaert J. and Grau Y. (1996) Cloning and functional expression of a drosophila metabotropic glutamate receptor expressed in the embryonic central nervous system. *J. Neurosci.* **16**:6687-6694.

9. Burstein E.S., Bräuner-Osborne H., Spalding T.A., Conklin B.R. and Brann M.R. (1997) Interactions of muscarinic receptors with the heterotrimeric G proteins Gq and G12: Transduction of proliferative signals. *J. Neurochem.* **68**:525-533.

10. Komatsuzaki K., Murayama Y., Gimabarella U., Ogata E., Seino S. and Nishimoto I. (1997) A novel system that reports the G-proteins linked to a given receptor: A study of type 3 somatostatin receptor. *FEBS Lett.* **406**:165-170.

11. Kostenis E., Gomeza J., Lerche C. and Wess J. (1997) Genetic analysis of receptor-Gaq coupling selectivity. *J. Biol. Chem.* **272**:23675-37681.

12. Monteclaro F.S., Arai H. and Charo I.F. (1997) Molecular approached to identifying ligand binding and signaling domains of c-c chemokine receptors. *Methods Enzymol.* **288**:70-84.

13. Tsu R.C., Ho M.K.C., Yung L.Y., Joshi S. and Wong Y.H. (1997) Role of amino- and carboxyl-terminal regions of Gaz in the recognition of Gi-coupled receptors. *Mol. Pharmacol.* **52**:38-45.

14. Coward P., Wada H.G., Falk M.S., Chan S.D.H., Meng F., Akil H. and Conklin B.R. (1998) Controlling signaling with a specifically designed Gi-coupled receptor. Proc. Natl. Acad. Sci. 95:352-357.

15. Ancellin, N., and Hla, T. (1999) Differential pharmacological properties and signal transduction of the sphingosine 1-phosphate receptors EDG-1, EDG-3, and EDG-5. *J. Biol. Chem.* **274**: 18997-19002.

I hope this information is useful. Please send preprints of papers that use the chimeras, and feel free to contact me if you have suggestions.

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For further information, see Conklin Lab Technical Information

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